<Division of Biochemistry>Biofunctional Design-Chemistry

Division of Biochemistry
– Biofunctional Design-Chemistry –

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Scope of Research

The ultimate goal of our research is the regulation of cellular functions by designed peptides and proteins. Current research subjects include (1) development of novel intracellular delivery systems aiming at elucidation and control of cellular functions using designed membrane permeable peptide vectors, (2) elucidation of the DNA or RNA binding modes of nucleic acid binding proteins, and design of artificial regulators of gene expression, (3) elucidation and control of membrane curvature, and (4) design of stimulation-responsible artificial peptides and proteins.

KEYWORDS

Membrane-Permeable Peptides
Intracellular Delivery
Peptide Design
DNA/RNA Binding Protein
Membrane Curvature

Selected Publications

Masuda, T.; Baba, K.; Nomura, T.; Tsujita, K.; Murayama, T.; Itoh, T.; Takatani-Nakase, T.; Sokabe, M.; Inagaki, N.; Futaki, S., An Influenza-derived Membrane Tension-modulating Peptide Regulates Cell Movement and Morphology via Actin Remodeling, *Commun. Biol.*, **2,** 243 (2019).

Akishiba, M.; Futaki, S., Inducible Membrane Permeabilization by Attenuated Lytic Peptides: A New Concept for Accessing Cell Interiors through Ruffled Membranes, *Mol. Pharm.*, **16,** 2540-2548 (2019).

Kawaguchi, Y.; Ise, S.; Azuma, Y.; Takeuchi, T.; Kawano, K.; Le, T. K.; Ohkanda, J.; Futaki, S., Dipicolylamine/Metal Complexes that Promote Direct Cell-Membrane Penetration of Octaarginine, *Bioconjug. Chem.*, **30,** 454-460 (2019).

Sakai, T.; Kawano, K.; Iino, M.; Takeuchi, T.; Imanishi, M.; Futaki, S., Loosening of Lipid Packing by Cell-Surface Recruitment of Amphiphilic Peptides by Coiled-Coil Tethering, *Chembiochem*, **20,** 2151-2159 (2019).

Akishiba, M.; Takeuchi, T.; Kawaguchi, Y.; Sakamoto, K.; Yu, H. H.; Nakase, I.; Takatani-Nakase, T.; Madani, F.; Gräslund, A.; Futaki, S., Cytosolic Antibody Delivery by Lipid-Sensitive Endosomolytic Peptide, *Nat. Chem.*, **9,** 751-761 (2017).

Artificial Curvature Inducing Peptide Triggering Cellular Endocytic Uptake

Membrane curvature is no longer seen as a passive consequence of cellular activity but an active means to create membrane domains and to organize centres for membrane trafficking (McMahon & Gallop, *Nature*, (2005) 438, 590-596). The generation and maintenance of membrane curvature is of central importance for maintaining trafficking and cellular functions. It is therefore meaningful to develop a new tool for controlling membrane curvature to understand the fundamental aspects in cell homeostasis and the disorder. As a new tool candidate, we focused on amphipathic peptides. Some amphipathic peptides are known to induce membrane curvature by its interaction with membrane. We hypothesized that these amphipathic peptides can also induce membrane curvature in living cells by the interaction with plasma membranes and control cellular functions.

As a cellular activity which was regulated by membrane curvature, we focused on endocytic events. Plasma membrane curvature controls endocytosis events by affecting the activities of endocytic proteins (Zhao *et al*., *Nat. Nanotech*., (2017) 12, 750-756). Therefore, we hypothesized that amphipathic peptides can control endocytic events by inducing membrane curvature in living cells (Figure 1). To confirm the effects on endocytic events by amphipathic peptides, we measured the cellular uptake amount of fluorescent-labelled dextran, which is a fluid-phase endocytosis marker. In the amphipathic peptide-treated cells, increase in cellular uptake of fluorescent-labelled dextran was observed. Amphiphysin is a membrane curvature-sensing protein. The treatment of the cells with the amphipathic peptide increased the number of amphiphysin-derived puncta signals, suggesting that the amphipathic peptide may induce membrane curvature in living cells.

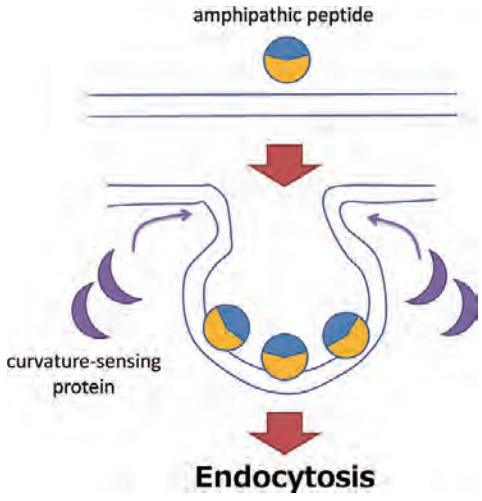

Figure 1. Mechanism of effect on endocytosis by amphipathic peptide.

Loosening of Lipid packing by Recruitment of Amphiphilic Peptides onto Cell Surface

Spatiotemporal membrane remodeling plays an important role in cellular events, including membrane trafficking, movement, growth and division. Alternations of the membrane structure are often accompanied by change of lipid packing. Establishment of means of controlling lipid packing may thus enable modification of various cellular functions and events.

Epsin-1 is an accessory protein involved in the induction of positive curvature necessary for clathrin-coated pit formation. We previously reported that an amphiphilic helical peptide corresponding to the N-terminus 18 residues (EpN18) has an ability to induce positive curvature[1] and to loosen lipid packing of cell membranes.[2] We have therefore focused on EpN18 as a means of manipulating lipid packing and the structure of the cell membranes.

In this study, we report a novel approach for recruitment of EpN18 onto cell surface using leucine-zipper peptides, E3 and K4, which form a stable heterodimer with a parallel coiled-coil structure.[3] EpN18 was conjugated to the K4 segment, which specifically recognizes E3 segments expressed on cell surface. Live-cell confocal laser scanning microscopy analysis revealed that this cell-surface tethering of EpN18 yielded promotion of intracellular translocation of octaarginine and loosening of lipid packing. In addition, detachment of EpN18 from cell surface was accomplished by the treatment of excess amount of the K4 peptide without bearing EpN18. This approach thus shows promise for the modulation of lipid packing and related cellular events.[4]

[1] S. Pujals *et al.* (2013) *ACS Chem. Biol.*, **8,** 1894-1899.
[2] T. Murayama *et al.* (2017) *Angew. Chem. Int. Ed.*, **56,** 7644-7647.
[3] Y. Yano *et al.* (2008) *ACS Chem. Biol.*, **3,** 341-345.
[4] T. Sakai *et al.* (2019) *ChemBioChem*, **20,** 2151-2159.

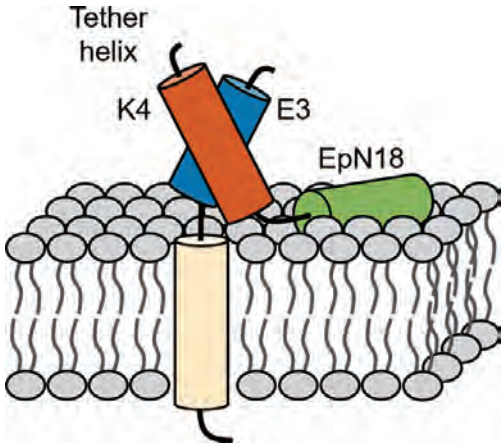

Figure 2. Tethering of EpN18 to cell-surface-expressed E3 segments by means of a coiled-coil heterodimer formation.